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Risk factors for elevated *Enterococcus* concentrations in a rural tropical island watershed

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ABSTRACT

Associations were examined between riparian canopy cover, presence of cattle near streams, and month of year with the concentration of *Enterococcus* (Most Probable Number (MPN)/100 ml) in surface water at Waipā watershed on the North Side of the Hawaiian island Kaua'i. Each one percent decrease in riparian canopy cover was associated with a 3.6 MPN/100 ml increase of waterborne *Enterococcus*. Presence of cattle near monitoring sites was associated with an increase of 99.3 MPN/100 ml of *Enterococcus* in individual grab samples. Lastly, summer samples (July) were substantially higher in concentration of *Enterococcus* than winter collected samples (February) in *Enterococcus* in sampled streams. These results suggest that reducing canopy cover and introduction of cattle into riparian zones may contribute to increases of *Enterococcus* concentrations in stream water.

1. Introduction

The World Health Organization (WHO) estimates that about 2.6 billion people do not use improved sanitation and that 884 million people do not use improved sources of drinking water (WHO, 2010). The vast majority of diarrhoeal disease in the world (88%) is attributable to unsafe water, sanitation and hygiene (WHO, 2003). Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (disability adjusted life years [DALYs]) world-wide (54.2 million) are attributable to unsafe water, sanitation and hygiene (Ashbolt, 2004). Infectious disease transmission may occur via surface waters when these waters become contaminated with fecal material from humans, livestock or wildlife (USEPA, 2003). A common procedure for monitoring microbial water quality is to measure the concentration of one or more fecal indicator bacteria (FIB) as proxies for the presence of fecal waste and waterborne pathogens. However, there are concerns about the use of FIB as proxies due to: 1) poor correlations between FIB and pathogens; 2) inability of FIB to differentiate sources; and 3) survival, growth and adaptation of FIB in the environment (Field and Samadpour, 2007). For example, high *Enterococcus* fecal indicator bacteria concentrations in water and soil samples collected in lower and mid-elevation tropical island watersheds could be a result of overpopulation, land degradation, and ongoing fecal input from feral animals and people (Fujioka et al., 1999; Hardina and Fujioka, 1991). Also, the unknown capacity for *Enterococcus* to survive and/or reproduce in warm, humid, tropical environments creates uncertainty about the reliability of *Enterococcus* as an indicator of fecal contamination and pathogen presence in surface water. Background environmental levels of *Enterococcus* in surface water collected on tropical ecosystems are potentially much higher than under conditions studied to establish USEPA individual sample *Enterococcus* guidelines in Boston Harbor, MA; New York City, NY; and New Orleans, LA (Shibata et al., 2004). But, some research shows fecal bacteria specifically indicate fecal contamination of tropical surface water (Gonzalez et al., 2010;

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Isobe et al., 2004), associates fecal bacteria with the anthropogenic influence status of tropical waters (Byamukama et al., 2005), and suggest that the species composition and antimicrobial resistance of *Enterococci* in tropical aquatic environments are influenced by fecal and antimicrobial pollution (Petersen and Dalsgaard, 2003).

However, other researchers claim that *Enterococcus* occurs naturally in tropical island soil and water producing false positive results with respect to implied contamination by feces of warm-blooded animals and the microbiological safety of water supplies; thereby making *Enterococci* invalid as an indicator of contamination by human and animal feces on tropical islands (Byappanahalli and Fujioka, 2004; Fujioka et al., 1999; Fujioka, 2001; Hardina and Fujioka, 1991). But, limited fecal bacteria soil and water quality data collected on lower to mid elevations of the overpopulated tropical islands of O'ahu, Guam, and Puerto Rico (Fujioka et al., 1999; Hardina and Fujioka, 1991; Hazen, 1988) creates concerns about claims regarding naturally occurring non-fecal soil and water sources of fecal bacteria. More recently, positive *enterococcal source protein* (*esp)* gene assays in the Hanalei watershed of Kaua'i indicate that some *Enterococci* in environmental samples were of human fecal origin (Knee et al., 2008). Also, the Almendares River, located in Havana city, Cuba receives the wastewaters of more than 200,000 inhabitants and the high abundance of fecal bacterial indicators (FBIs) in the downstream stretch of the river reflects the very poor microbiological water quality (Garcia Armisen et al., 2008). Researchers also found that recreational activity resulted in reduced fecal bacteria water quality in tropical island stream water: sites with recreation had poorer fecal bacteria water quality than those without; water quality was generally poorer when there were high numbers of recreational users (Phillip et al., 2009).

Furthermore, *Enterococci* concentrations (Most Probable Number (MPN)/g) in rural tropical island soils were not significantly associated with *Enterococci* geometric mean concentrations (MPN/100 ml) in the same rural tropical island stream water (Ragosta et al., 2010). Plus, reducing tropical island stream tree canopy was significantly associated with increases in geometric mean of *Enteroccoci* in stream water (Ragosta et al., 2010).

U.S. Environmental Protection Agency (USEPA) recommends testing for *Enterococcus* for fresh and marine waters because presence of *Enterococcus* in water samples that exceed USEPA guidelines were directly correlated with gastrointestinal illness rates associated with recreational contact (USEPA, 1986). A single grab sample cannot exceed 104 *Enterococci* Most Probable Number (MPN)/100 ml in marine waters (USEPA, 1986). In fresh water no single grab sample should exceed a one sided confidence limit (C.L.) calculated using the following criteria as guidance (Designated bathing beach 75% C.L., Moderate use for bathing 82% C.L., Light use for bathing 90% C.L., Infrequent use for bathing 95% C.L.) based on a site-specific $\log_{10}$ standard error, or if site data are insufficient to establish a $\log_{10}$ standard error, then using .4 ($n = 5$) as the $\log_{10}$ standard error for *Enterococcus* (USEPA, 1986). Given individual sample *Enterococcus* recommendations by USEPA, 1986, relationships were examined between *Enterococcus* individual water sample concentrations with riparian canopy cover and presence or absence of cattle on the Hawaiian island of Kaua'i (Fig. 1) for monitoring and regulatory enforcement based upon multiple grab sample collections per month at each site (Fig. 2) during 2004 and 2005. Evaluation of *Enterococcus* as a reliable indicator of individual samples of surface water fecal contamination is required to establish that this monitoring metric is effective for protecting human health and guiding implementation of water quality management practices.

2. Objectives

This study evaluated the association between the presence of cattle near streams, reductions in riparian canopy cover and *Enterococcus* concentrations in water samples from Waipā Stream on the Hawaiian island of Kaua'i. A significant association, either positive or negative, would be important for developing a sampling strategy that would be effective in assessing the risk of fecal

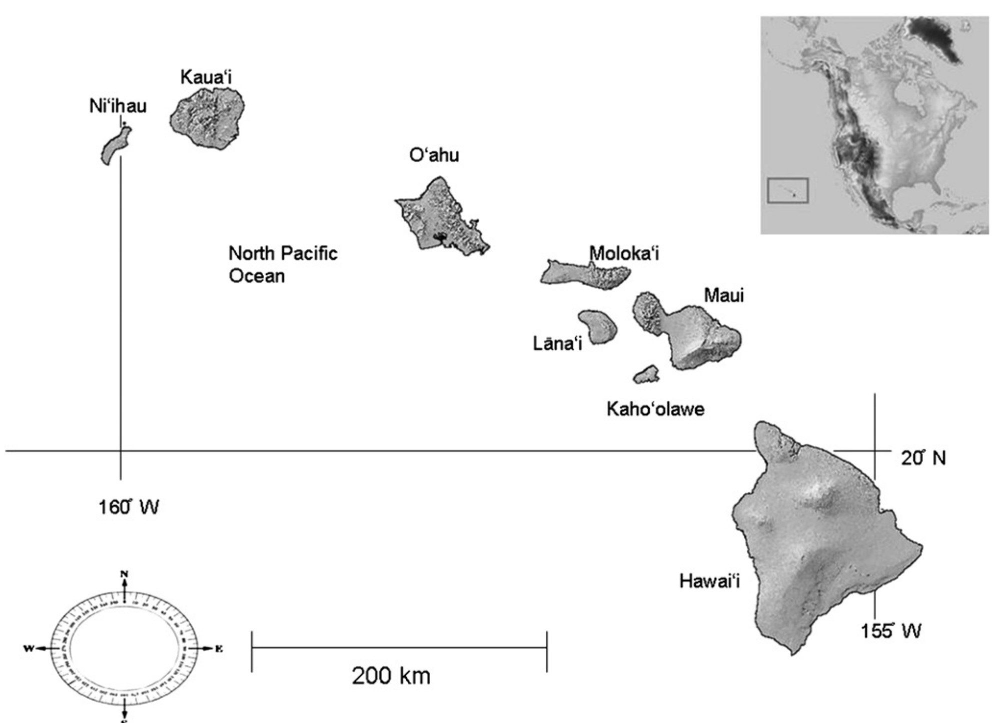

Fig. 1. Map of Hawaiian Islands.

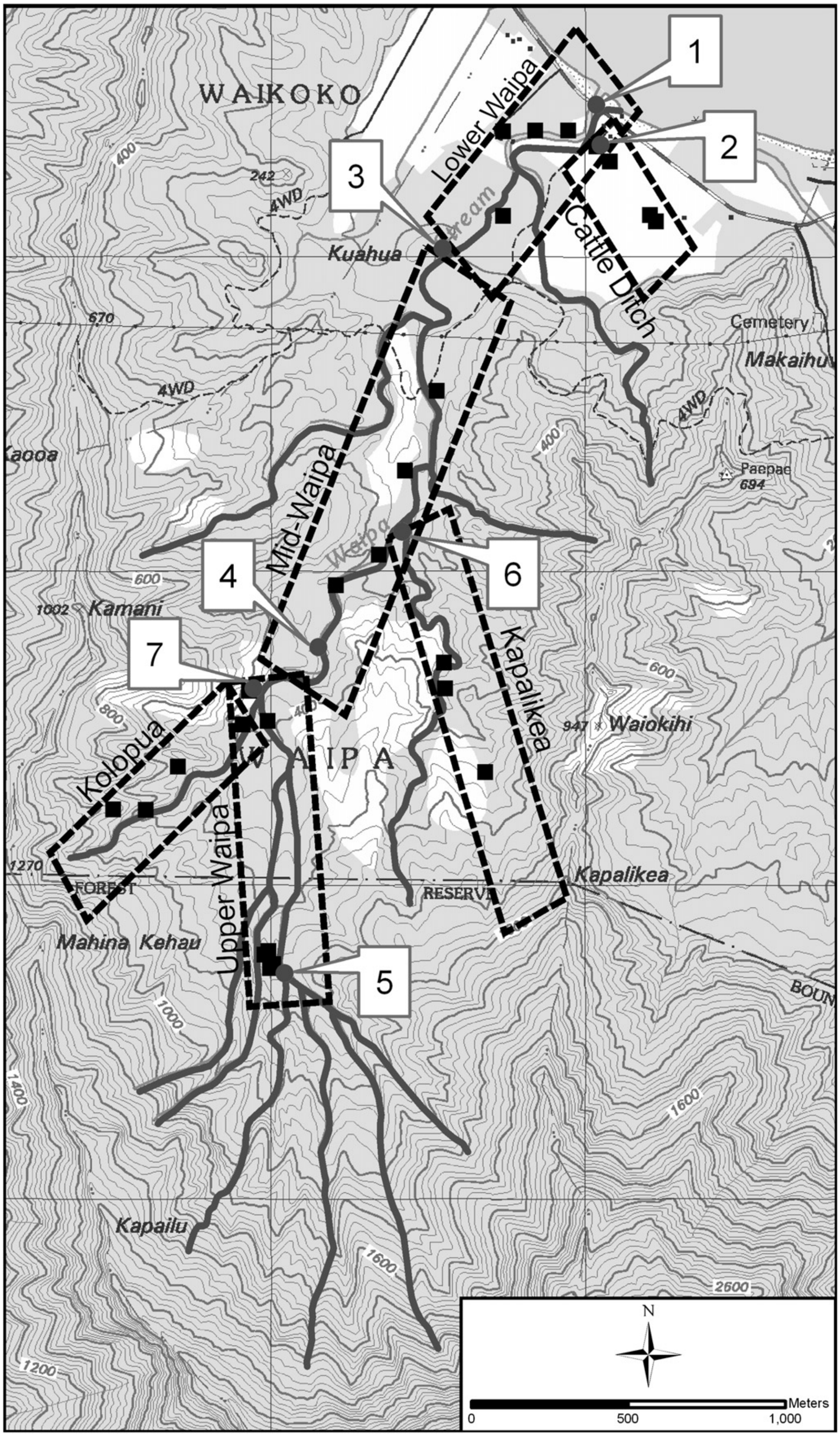

Fig. 2. 24 Plot(■) and 7 water quality monitoring sites(●). There are 24 plots (4 plots/section). Some plots are superimposed due to proximity of the plots. Canopy cover was measured in all sections from site 5 down to site 1 near MSL.

contamination from watershed activities. Furthermore, a significant positive association ($P < .05$) between a factor such as cattle presence near streams and individual water sample concentrations of *Enterococcus* (MPN/100 ml) would strengthen the assertion that this indicator organism is a suitable and sensitive indicator of how land use practices can influence microbial quality of stream water. It was also determined if there were monthly or seasonal shifts in the concentration of *Enterococcus* to better understand natural fluctuations of indicator bacteria in a tropical watershed.

3. Methods

3.1. Study site description

Waipā watershed encompasses about 650 ha from near sea level to Mamalahoa Summit at about 1141 m above Mean Sea Level (MSL). Lower to mid-elevation Waipā riparian zones previously contained cattle moved throughout the lower watershed elevations into areas around mid-elevation (about near sea level to around 200 m above sea level) until the 1960s. Cattle are now paddocked near MSL. Lower elevation Waipā includes ditches used to irrigate vegetables and fruit trees, several residences, Kamehameha Highway, a community center, and about 50 cattle and a few horses that graze an approximately 2 ha paddocked pasture. Cattle grazed along Kapalikea tributary until the late 1960s when land managers confined remaining cattle around sites 1, 2, and 3 (Fig. 2). During the study period, most of the approximately 50 cattle congregated and grazed daily within <100 m of sites 1 and 2 within the approximately 2 ha paddocked area, with a lesser density of cattle straying outside the paddocks to <100 m of site 3 (100 m was approximately the closest distance that cattle were ever observed near site 3). Cattle were never present at sites 4, 5, 6, or 7 due to confinement and active management, and terrain too difficult for cattle to access anywhere above site 3. An irrigation diversion ditch runs along the cattle pasture near MSL and drains surface runoff from site 2 into Waipā Stream mouth at site 1, which exits directly into the Pacific Ocean.

3.2. Site and plot design

Twenty-four randomly located riparian canopy monitoring plots, and seven stream *Enterococci* water quality monitoring sites (Fig. 2) were established. Riparian canopy cover (%) was measured and species composition was characterized in each plot (see below). Each of 7 stream water quality monitoring sites (Fig. 2) were established to collect individual water samples at the end of a cattle pasture diversion ditch ($n = 1$), alongside stratified elevation and canopy sections of Waipā Stream ($n = 4$), and at the confluence of Kapalikea ($n = 1$) and Kolopua ($n = 1$) tributaries above Waipā Stream (Fig. 2). Cattle were observed daily approximately 100 m from stream monitoring site 1, within 5 to <100 m from site 2, and <100 m from site 3 daily from June 1, 2004 to August 31, 2004, and daily from January 1, 2005 to August 1, 2005. Neither cattle, nor individual cattle sign were ever observed within approximately 2000–3000 m of sites 4, 5, 6, and 7 during all site visits throughout the same monitoring periods for cattle at sites 1, 2, and 3.

3.3. Stream Enterococcus concentration monitoring site data collection

Individual stream water samples ($n = 3$) from each of 7 stream monitoring sites were collected on July 9 and 23 2004, February 9, March 9 and 23, and June 1 2005 at approximately 8:30 a.m. Hawaii time (APHA, 2005). Each individual sample was analyzed for *Enterococcus* using the quantitative Enterolert IDEXX method (USEPA, 2003). Water samples were diluted 10 to 1 (90 ml:10 ml) with a sterile buffered solution February 9, 2005, March 9, 2005, and March 23, 2005 due to concerns that increased fecal surface runoff during the historically wet season would exceed the upper detection limit (2419.6 MPN/100 ml) of the analytical procedure. Water samples collected during the historically dry season (July 9, 2004, July 23, 2004, and June 1, 2005) were not diluted based on the assumption that *Enterococcus* levels would not exceed 2419.6 MPN/100 ml due to decreased surface runoff events. If *Enterococcus* (MPN/100 ml) concentrations were <1 MPN/100 ml, a surrogate value of .1 MPN/100 ml was used for statistical analysis of individual samples.

3.4. Riparian monitoring plot canopy cover data collection

Within each of the 24 riparian monitoring plots, three transects were oriented parallel to the stream channel at the stream bank edge, midway, and at the uppermost edge of the plots. Canopy cover was measured in each plot two days/week in July and August 2004 using a densitometer every 50 cm along each transect, for a total of 63 observations of canopy cover per 10×10 m plot, and 33 observations of canopy cover per 5×5 m plot. Tributary plot sizes were adjusted to 5×5 m due to reduced riparian area size. Riparian plots were adjacent to stream monitoring sites at the end of stratified sections along Waipā Stream, at the confluence of two tributaries directly above Waipā Stream, and at the end of a cattle pasture irrigation diversion ditch directly above Waipā Stream mouth to determine how different nearby sections of land use are associated to adjacent *Enterococci* surface water concentrations.

3.5. Statistical analysis

Linear mixed effects regression analysis was used to identify significant associations between cattle presence or absence and canopy cover associations ($P < .05$) and the concentration of *Enterococcus* (MPN/100 ml) in adjacent individual stream water samples. Linear mixed effects regression analysis (Pinheiro and Bates, 2000) is a statistical model well suited to water quality and other environmental research where investigators repeatedly sample a set of locations and want to determine if individual water samples are significantly associated with one or more land use patterns, climate measurements, geomorphological attributes, or other such independent factors operating at the plot-, field-, or watershed-scale (Atwill et al., 2006; Ragosta et al., 2010; Tate et al., 2006). The results of the model are similar in many ways to linear regression, except that in mixed models the coefficients, *P*-values, and 95% confidence intervals are adjusted for the amount of correlation within the dataset attributable to repeated sampling at a set of sites, using a repeated measures adjustment (Pinheiro and Bates, 2000).

Using linear mixed effects regression, it was determined if canopy cover (%) at the plots, and presence of cattle near the stream monitoring site were associated ($P < .05$) with the concentration of *Enterococcus* (MPN/100 ml) in adjacent individual stream water samples (Pinheiro and Bates, 2000). These various physical and plot-specific factors functioned as the independent variables, concentration of *Enterococcus* (MPN/100 ml) in individual water samples was the dependent variable, and sample site functioned as the group variable. Given that three separate stream water samples were collected in triplicate at each site for each sampling event (e.g., 3 consecutive water samples) and *Enterococcus* was measured for each of the three samples, the regression model was built by treating each individual water sample separately in the statistical

Table 1
Linear mixed effects regression model of canopy cover (%), month, and presence of cattle on the concentration of *Enterococcus* (MPN/100 ml) in stream water in Waipa watershed during 2004 and 2005.

Factor	Coefficient[a]	95%CI[b]	P value[c]
Month			
February[d]	0.0	–	–
March	16.6	(−122, 156)	0.810
June	18.9	(−142, 179)	0.813
July	204	(65, 343)	0.005
Canopy Cover %	3.6	(−4.7, −2.0)	<0.0001
Cattle			
Absent[d]	0.0	–	–
1 or more present	99.2	(4.1, 194)	0.041
Intercept	294	(103, 184)	0.0004

[a] Total *Enterococcus* (MPN/100 ml) per site during the study period of 2004 and 2005 was set as the dependent variable; month, canopy cover %, and cattle presence were set as fixed independent effects. Site identity set as a group effect to account for repeated measures. Coefficients are for total *Enterococcus* (MPN/100 ml) values.
[b] CI, confidence interval.
[c] Significance (coefficient not equal to 0) was determined by $P < .05$ using a conditional *t* test.
[d] Referent condition to which other levels of the categorical factor are compared.

analysis, given that they were separate water samples. The advantage of analyzing all individual samples of *Enterococcus* concentrations in stream water is that it results in a larger sample size than using the geometric mean. A forward-stepping approach was used to develop a final model, with $P \leq .05$ set as the criterion for inclusion of the independent variables into the final model based on a conditional *t*-test (Pinheiro and Bates, 2000). Assumptions of normally distributed error terms and constant variance in error terms were confirmed by graphical evaluation of standard diagnostic plots.

4. Results, discussion, and conclusion

Month ($P = .005$), canopy cover % ($P < .0001$), and presence of cattle ($P = .041$) were significantly associated with concentration of *Enterococcus* (MPN/100 ml) in stream water samples (Table 1). Presence of cattle within 100 m of stream monitoring sites was associated with an increase in *Enterococcus* of 99 MPN/100 ml (Table 1). Each one percent decrease in riparian canopy cover was associated with a 3.6 MPN/100 ml increase in *Enterococcus* in stream water (Fig. 3). Lastly, water sampling in July compared with February was associated with an increase of 204 MPN/100 ml of *Enterococcus* in the streams of the Waipā watershed. The linear mixed effects regression describing these associations can be evaluated by the

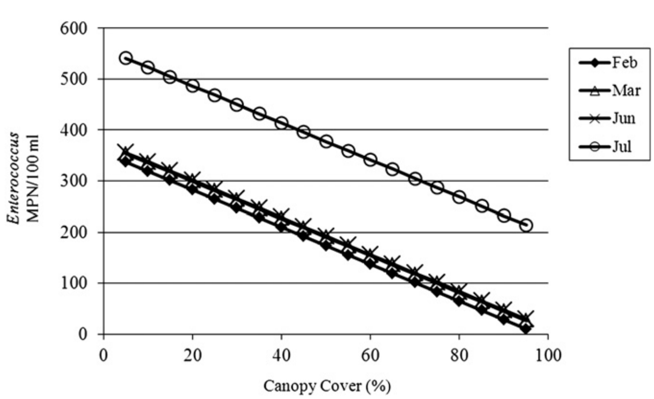

Fig. 3. Predicted *Enterococcus* (MPN/100 ml) concentrations of individual samples as a function of canopy cover (%) and month.

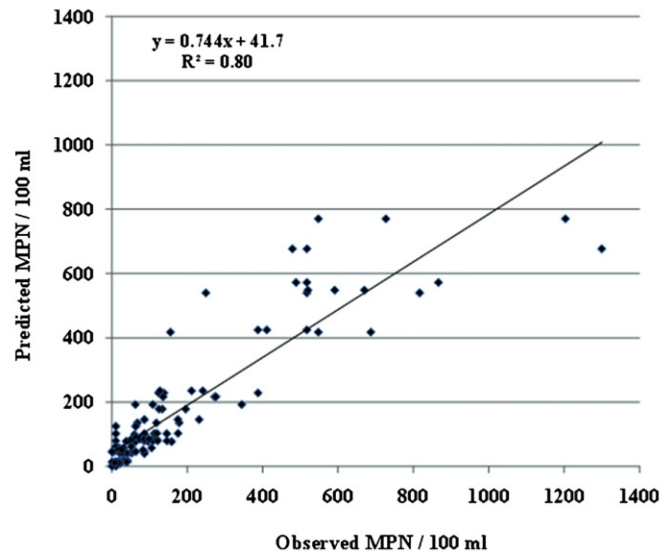

Fig. 4. Observed versus predicted grab sample *Enterococcus* concentrations (MPN/100 ml), as calculated by linear mixed-effects model containing independent variables of canopy cover, month, and presence of cattle near monitoring sites.

comparison of observed versus predicted data for the concentration of *Enterococcus* (MPN/100 ml) in individual water samples (Fig. 4). In general, the regression model slightly overestimates *Enterococcus*at lower concentrations and slightly overestimates at the higher concentrations, indicating that model predictions need to be interpreted with caution.

Researchers have proposed that soil is a major environmental source of *Enterococcus* in samples collected and analyzed from tropical islands. Several studies have also noted uncertainties about the suitability of individual grab samples of *Enterococcus* (Fujioka et al., 1999; Hardina and Fujioka, 1991) as a fecal indicator in tropical island ambient waters. The primary concern is that *Enterococcus* may be naturally occurring in tropical island soil environments (Fujioka et al., 1999; Hardina and Fujioka, 1991), rather than from fecal contamination from an animal or human. But, presence of cattle near sites 1 and 2 (Fig. 2), lowest canopy cover % along the cattle ditch section (Fig. 2), irrigation water transport through site 2 to site 1, and the presence of residences above site 1 may all contribute to the higher concentrations of *Enterococci* results at site 1.

Reduced canopy cover was associated with an increase, not decrease in *Enterococci*, possibly due to increased erosion in riparian zones with the lowest % canopy cover along Kapalikea tributary and the cattle pasture irrigation diversion ditch (Table 2), and transport potential of exposed soil surfaces compared to sites protected by almost 100% canopy.

Table 2
Arithmetic mean for *Enterococcus* concentrations at stream monitoring sites with and Without cattle, at different months, and at stratified canopy cover values.

Cattle	*Enterococcus* concentrations (MPN/100 ml)	Standard deviation
Absent	101.7	186.8
Present	244	271
Month		
February	86.1	62.8
March	102.7	148.7
June	104.9	227.9
July	289.8	310.2
Canopy Cover		
0–49%	348.5	345.1
50–100%	88.3	110.5

Continued research on movement, reproduction, and survival of fecal bacteria and pathogens through tropical watersheds could improve knowledge about the efficacy of guidelines established for microbial water quality. Identifying how *Enterococcus* enters near shore waters will help environmental managers address pressing water quality issues, including the significance of exceeding *Enterococcus* water quality individual sample standards (Knee et al., 2008). Given concerns that *Enterococci* monitoring and recording of individual samples for statistical analysis in fresh and marine waters could falsely indicate fecal contamination under USEPA guidelines, it would help to determine their longevity, particularly in conditions found on rural tropical islands. If the source of *Enterococcus* in individual water samples is feces, further research is needed to determine how *Enterococcus* from feces can contaminate food, water, and the tropical environment for people, domestic and feral animals. Data from this research indicated that *Enterococcus* water sample concentrations increased by decreasing riparian canopy cover % and allowing cattle to enter stream corridors of a rural tropical island. Perhaps *Enterococcus* concentrations in individual water samples increases due to overgrazing, reduction of canopy cover in the riparian zone, cattle defecating directly in the stream, increased fecal surface runoff, and soil compaction. It is possible that previous contamination, followed by degradation and disappearance of the fecal matrix, may leave *Enterococci* in tropical island soils and waters even though individual fecal samples do not appear to be present.

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